ASSESSMENT OF THE GLYCAEMIC INDEX, CONTENT OF BIOACTIVE COMPOUNDS, AND THEIR *IN VITRO* BIOACCESSIBILITY IN OAT-BUCKWHEAT BREADS.

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Abstract

This study addressed determinations of the glycemic index, antioxidant capacity, and phenolics content of oat, buckwheat, and mixed oat/buckwheat breads. The bioaccessibility of total phenolic compounds and the antioxidant capacity of breads were studied after *in vitro* digestion. The lowest values of the glycaemic index were determined for oat bread, whereas breads with

the highest content of buckwheat flour had the highest antioxidant capacity. The digestion of breads showed that most of the phenolic compounds which exhibit the antioxidant activity were soluble in the digestive fluid as their high content was found in the soluble fraction. Noteworthy is that the phenolic compounds were still present in the insoluble fraction after digestion, and could be available for intestinal microflora. It was concluded that the mixed oat-buckwheat breads may serve as products with a medium glycaemic index, as a source of phenolic compounds, and as products with a high antioxidant activity, which could be potentially enhanced by enzymatic digestion of fermentation by microbiota.

Keywords: oat, buckwheat, antioxidant, glycaemic index

1. Introduction

Bread is the most widely used food product in the world, that is why its sensory but also nutritious or technological quality is of the utmost importance. However, let us not forget about consumers on a gluten-free diet, including these suffering from coeliac disease, noncoeliac gluten sensitivity or wheat allergy. Development of gluten-free products involves a multifaceted approach entailing the use of substances that could improve their structure, mouthfeel or acceptability. As demonstrated by Saturni et al (2010), 20-38% of the coeliac patients have some nutritional deficiencies of calories/protein, dietary fiber, minerals or vitamins. Therefore, the optimization of product recipes, including bread, and characterization of final products in terms of their sensory acceptance and potential health-promoting components (such as fibre, antioxidants or minerals) still capture much attention.

Buckwheat and oat products have been addressed in many scientific studies that were intended to confirm their functional properties (Behall and Hallfrisch, 2011; Giménez-Bastida and Zieliński, 2015). Due to the increasing amount of information available, both buckwheat

and oats, find a wider variety of applications: in dietetics, medicine, pharmaceutical industry or cosmetics (Wronkowska et al., 2010a; Webster, 2011). Bread enrichment , for example, bioactive compounds or those with a documented impact on human health, can positively affect consumer health. Both buckwheat and oats have already found application in bakery products, especially as components of wheat bread, or in gluten-free products (Wang et al., 2017; Verardo et al., 2018). As presented by Zhang et al. (2012), buckwheat is a good source of nutritionally valuable protein, lipid, dietary fibre, and minerals; it is also known for its bioactive components. The mechanisms underlying the beneficial effects attributed to selected buckwheat bioactive compounds (such as flavonoids, proteins or *D*-chiro-inositol) were described in the review by Giménez-Bastida and Zieliński (2015). Based on human, animal, and *in vitro* studies, the health benefits attributed to buckwheat bioactive compounds include: plasma cholesterol level reduction; neuroprotection; as well as anticarcinogenic, anti-inflammatory or antidiabetic effects. In turn, health benefits of oats were presented in the review by Martínez-Villaluenga and Peňas (2017). These authors showed that such components as β-glucan, avenanthramides, tocols, sterols, phytic acid, and avenacosides could be involved in reducing the risk of development of cardiovascular diseases, type 2 diabetes mellitus, gastrointestinal disorders or cancer. Therefore, the mixed oat/buckwheat products seem to be very attractive to consumers as their bioactive components are highly bioaccessible.

Due to the increasing interest in the health-promoting properties of food, antioxidants are in the focus of interest of researchers and consumers. As reported by Halliwell et al. (1995), antioxidants occur naturally in plants in the form of secondary metabolites, which primarily fulfill a protective function for a plant. Some epidemiological surveys have shown some health benefits to be positively correlated with the consumption of plant-derived foods (Espin et al., 2007). However, noteworthy is the “antioxidant paradox” resulting from human intervention studies, which failed to demonstrate the preventive or therapeutic effect of large doses of dietary

antioxidant supplementation (Halliwell, 2013; Niki, 2016). An antioxidant entrapped in the structures of the food matrix could be released during the gastrointestinal digestion. But, of course, a part of antioxidant molecules may still be entrapped in the non-digestible fraction and after interaction with microflora they, or their metabolites, could be released and absorbed in the last segment of the digestive tract. During the intestinal digestion, ca. 48% of dietary polyphenols remain bioaccessible in the small intestine, about 42% are still bioavailable in the large intestine, but about 10% never leave the food matrix during the digestion process (Saura-Calixto et al., 2007).

The main objective of this research was to determine the optimal formulation of mixed oat/buckwheat breads in terms of their biological properties, level of antioxidant compounds, and capability to lower the glycaemic index. Additionally, the *in vitro* enzymatic digestion of breads was conducted to determine whether phenolic compounds and antioxidants are released from the food matrix.

2. Materials and Methods

2.1. Chemicals and reagents

Reagents used in the assays of antioxidant properties and all enzymes used in the *in vitro* digestion and glycemic index analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were from Avantor Performance Materials Poland S.A. (Gliwice, Poland). Water was purified with a Mili-Q-system (Milipore, Bedford, USA).

2.2. Materials

Commercially available buckwheat (*Fagopyrum esculentum* Moench) and oat (*Avena sativa* L.) flours were purchased from a local producer (Melvit S.A., Kruki, Poland). According to producer's declaration, contents of carbohydrates, proteins, ash, and fat in buckwheat and

oat flours were: 65.2 and 60.4%; 19.2 and 15.4%; 3.2 and 2.1%; and 0.7 and 7.1% of flour dry matter, respectively.

2.3. Breadmaking process

The bread dough formula consisted of both analysed flours in different proportions: 600 g of oat flour (O); or 600 g of buckwheat flour (B), or 480 g of oat flour and 120 g of buckwheat flour (OB20%); or 300 g of oat flour and 300 g of buckwheat flour (OB50%); or 120 g of oat flour and 480 g of buckwheat flour (OB80%). Additionally, 60 g of compressed yeast (*Saccharomyces cerevisiae*, Lesaffre Poland, Poland) and 8 g of sodium salt were used. Based on water absorption properties of flour determined with a Farinograph, the quantity of water used for dough preparation was as follow: 120 g/100 g of flours for O, B and OB50% formulas and 104 g/100 g of flours for OB20% and OB80% formulas. A GM-2 type mixer (ZBPP, Bydgoszcz, Poland) was used for 3-min mixing of all ingredients. The dough was proofed at 37^{o}C and 80% relative humidity for 60 min with puncture after 30 min. Afterwards, the whole dough was divided into 250-g portions and proofed up to optimum volume increase (about 30 min, 37^{o}C, 80% relative humidity) and baked at 220^{o}C for 50 min in a DC-21 model electric oven (Sveba Dahlen AB, Fristad, Sweden) with an incorporated proofing chamber.

2.4. In vitro digestion of bakery products

Delgado-Andrade et al. (2010) assay was used as a model of *in vitro* digestion; the protocol included three steps of digestion: saliva (α-amylase; pH 7.0; 30 min at 37°C), gastric (pepsin; pH 2.0; 2 h at 37°C), and intestinal (pancreatin and bile salts; pH 7.5; 2 h at 37°C). The enzymes were inactivated by heating (4 min at 100°C), the soluble and insoluble fractions obtained after centrifugation were used for further analysis. Insoluble fractions were freeze-dried and powdered to particles < 400 μm, while soluble fractions were used in the liquid form.

2.5. *Analysis of total phenolic compounds (TPC) content and antioxidant capacity in flours or bakery products before digestion*.

TPC were extracted from flours or freeze-dried breads with 80% MeOH and mixed with the Folin-Ciocalteu reagent. Absorbance of the extracts was measured at 725 nm and results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry matter (d.m.) of the sample (Zieliński et al., 2017). The antioxidant capacity of the 80% MeOH extract from flour or freeze-dried breads was determined against $ABTS^{\bullet+}$ radical cation and measured at an absorbance wavelength of 734 nm (Zieliński et al., 2017). The FRAP assay was performed according to Horszwald and Andlauer (2011) to analyse the ferric reducing ability of the experimental samples. The antioxidant capacity against superoxide anion radicals ($O_2^{-\bullet}$) was measured with the photochemiluminescence (PCL) method according to protocols for the determination of the antioxidant capacity of water-soluble antioxidants (ACW) (Analytik Jena, Germany). All results were expressed as μmol Trolox/g of sample d.m. (Zieliński et al., 2017).

2.6. *Analysis of total phenolic compounds (TPC) content and antioxidant capacity in bakery products after digestion*.

Soluble fractions (liquid samples). The content of TPC and antioxidant capacity ($ABTS^{\bullet+}$, FRAP and PCL ACW) of the soluble fraction, obtained after centrifugation of digested bread samples, were analyzed directly in the liquid sample using the methods described earlier (without previous extraction).

Insoluble fractions (freeze-dried samples). The total content of bioaccessible phenolic compounds (TPC-QUENCHER method) and bioaccessible antioxidant capacity (ABTS-QUENCHER method) of the insoluble fraction obtained after bread digestion were determined according Szawara-Nowak et al. (2016). The yields of the insoluble fraction, obtained after

digestion, for O, B, OB20%, OB50% and OB80% were: 15.8, 16.4, 16.5, 16.9, and 16.9%, respectively.

2.7. In vitro starch digestion and glycaemic index (GI) estimation

In vitro starch digestion and GI estimation were made according to Sanz-Penella et al. (2014). The rate of starch digestion was expressed as the percentage of total starch hydrolysed at 0, 20, 40, 60, 90, 120, and 180 min. The total starch content was evaluated using a commercial enzymatic kit (Total Starch Assay Procedure; Amyloglucosidase/α-Amylase Method; K-TSTA 07/11; Megazyme, Ireland). The GI value was calculated from the area under the hydrolysis curve (0 to 180 min) and total digestible starch; results obtained were normalised against white bread (SigmaPlot software, Version 12.0).

2.8. Statistical analysis

Two replications of all types of breads were prepared (3 loaves in each). All analyses were performed in three independent measurements, and all results are given as the means and the standard deviation. All data were analysed using one-way ANOVA (STATISTICA for Windows, StatSoft Inc., Tulsa, OK, USA, 2001) with Fisher's Least Significant Difference (LSD) post-hoc comparison at a significance level of $p<0.05$.

3. Results and discussion

The antioxidant capacity of flours and breads (Table 1 and 2) was analysed based on the results obtained by determining: TPC (total phenolic compounds) content, FRAP (ferric ion reducing antioxidant parameter), ABTS (against $ABTS^{+\bullet}$ radical cation), and PCL ACW (against superoxide anion radicals, $O_2^{-\bullet}$). The buckwheat flour (BF) had approximately 5-times higher content of TPC compared to oat flour (OF) and showed significantly higher antioxidant

capacity analysed with: FRAP, ABTS, and PCL assays (Table 1). As presented by Zieliński and Kozłowska (2000), the antioxidant activity of methanolic extracts from whole grain of the selected cereals was as follows: buckwheat > barley > oat > wheat = rye. Whereas Kaur et al. (2015) found that the total phenolics content was higher in wheat flour than in oat flour. Approx. 2.5-fold higher content of phenolic acids in barley whole grain flour compared to oat flour was determined by Hole et al. (2012).

Compared to the raw materials (oat and buckwheat flours), the content of compounds with antioxidant properties decreased after dough and bread making processes (Table 1 and 2). Among all analysed breads, those with the highest content of buckwheat flour (B and OB80%) had a significantly higher content of TPC (1.27 and 1.15 mg GAE/g d.m., respectively) (Table 2). The 20 and 50% addition of buckwheat flour to oat flour, in the recipe of OB20% and OB50%, caused approx. 3- and 6-fold increase in the TPC content compared to oat bread (O). Likewise for TPC, the content of BF in bread significantly increased its antioxidant capacity (Table 2). However, the 20% content of oat flour in the recipe of OB80% bread did not significantly decrease results obtained by PCL ACW assays compared to the bread made of 100% of buckwheat flour (B). While 20% content of buckwheat flour in the recipe of OB20% bread significantly increased its antioxidant capacity by approx. 1.5-times compared to oat bread (O). Even more evident enhancement of these properties (approx. 2-fold) was demonstrated for the bread containing 50% of buckwheat flour (OB50%) compared to oat bread (O).

Technological processes employed in the preparation of food products, e.g. fermentation or baking, and also heat treatment used in gastronomy, affect the content of biologically-active compounds. As reported by Hole et al. (2012), the fermentation of barley and oat flours with probiotic lactic acid bacteria strains significantly increased the content of free phenolic acids. Thermal treatment, as for example roasting process of buckwheat groats, significantly degraded

naturally occurring compounds exhibiting antioxidant properties, as found by Zieliński et al. (2009). Buckwheat is a very good source of compounds with antioxidant properties, as proved by Zieliński and Kozłowska (2000), therefore, it is often used as a food ingredient. As shown by Lin et al. (2009), wheat bread enriched with buckwheat flour had a significantly higher antioxidant potential compared to the control wheat bread. Also Wronkowska et al. (2010b) demonstrated a significant increase in the antioxidant potential of gluten-free breads with an increasing amount of buckwheat flour in the recipe.

The *in vitro* procedure for the evaluation of the rate of starch hydrolysis, expressed as a glycaemic index (GI), was used as a predictor of the physiological effect of food. The values of GI obtained for the examined breads are presented in Table 2. Breads made of 100% (O) or 80% of oat (OB20%) had statistically significantly ($p<0.05$) the lowest values of GI (69 and 70, respectively). In contrast, the highest GI values (72) were estimated for the breads prepared from 50% and 100% of buckwheat flour (OB50% and B, respectively). The oat-buckwheat breads examined in our study can be considered as food with intermediate GI, because as presented by Atkinson et al. (2008), food products can be differentiated into those with low (<55), intermediate (55-70), and high (>70) GI. In the international table of glycaemic index, Atkinson et al. (2008) showed a GI for buckwheat and oat breads to range from 63 to 95, but almost all of them were prepared with wheat flour addition. Compared to the results obtained in our study, Wolter et al. (2013) showed higher values of the predicted GI of buckwheat and oat gluten-free bread (80 and 71, respectively). As demonstrated by Thondre (2013), the factors which could affect the GI values are: differences in starch properties, methods used during processing, compactness and viscosity of bread or possible interactions with other food components that could impact starch digestibility. Scazzina et al. (2009) showed that sourdough fermentation could reduce the glycaemic response to wheat bread. However, as presented in the

review by Giuberti and Gallo (2018), the use of sourdough or flours from pseudocereals, like quinoa or buckwheat, does not always guarantee obtaining a product with a reduced GI.

The content of TPC and antioxidant capacity before and after *in vitro* digestion in the experimental breads are shown in Table 2. A significant increase was observed in the TPC content in the soluble and insoluble fractions compared to the samples before digestion (Table 2). Gawlik-Dziki et al. (2009) noticed the successive release of phenolic compound during the *in vitro* hydrolysis of wheat bread enriched with an extract from the green parts of buckwheat plant. An increase in TPC content was also shown by Szawara-Nowak et al. (2016) in the fractions obtained after *in vitro* digestion of buckwheat-enriched wheat breads. In turn, Liyana-Pathirana and Shahidi (2005) demonstrated a significantly increased TPC content after *in vitro* digestion of extracts from wheat whole grains and their flour, germ, and bran fractions.

The antioxidant capacity of the bread samples measured with the ABTS, PLC ACW, and FRAP assays before and after digestion under conditions simulating these occurring in the gastrointestinal tract are shown in Table 2. Because of methodological limitations, analyses of the ability to scavenge superoxide anion radicals (PCL ACW) and ferric reducing ability (FRAP) were not performed in the insoluble fraction.

Still little data is available in the literature on the effect of food digestion on the bioavailability of phenolic compound or components exhibiting antioxidant properties. For all analysed breads, a significant increase of the antioxidant activity after *in vitro* digestion was observed in both fractions (Table 2). The ABTS values were significantly higher in the soluble fraction (about 4-5 times), compared with the insoluble fraction. Also, about 1.5 to 3.3-times higher values of PCL ACW and FRAP were obtained for the soluble fraction, compared to the breads before digestion. Baublis et al. (2000) found an increase in the antioxidant activity in an aqueous extract from wheat-based cereals under the influence of pH treatment simulating gastrointestinal tract conditions. These authors suggested that the antioxidant activity of ~~t~~such

products could be enhanced by gastrointestinal conditions. The *in vitro* digestion is a crucial step that releases high amounts of phenolic antioxidants from the food matrix, as presented by Szawara-Nowak et al. (2016) for buckwheat-enriched wheat bread. Lafarga et al. (2019) also found that a higher amount of phenolic and antioxidant compounds could be released under the conditions simulating gastrointestinal digestion.

4. Conclusion

The antioxidant properties and the glycaemic index of bakery products obtained from oat and buckwheat flour were analysed. The breads examined were also subjected to *in vitro* digestion, and the bioaccessibility of their phenolic compounds and antioxidants was studied. The evaluation of the antioxidant properties of bakery products confirms that buckwheat flour is a carrier of a significantly higher amount of compounds exhibiting antioxidant activities. All analysed oat-buckwheat breads could be considered as food with intermediate glycaemic index, whereas bread obtained from 100% of oat flour had the lowest GI value. The presence of bioactive compounds in both fractions obtained after the *in vitro* digestion, especially in the insoluble one, may indicate a different extent of these compounds release from the food matrix.

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Conflict of interest

The authors declare that they have no conflict of interest.

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370 Table 1 The antioxidant capacity of oat and buckwheat flours.

371

	OF	BF
TPC [mg GAE/g d.m.]	0.63±0.06b	3.13±0.18a
ABTS [μmol Trolox/g d.m.]	1.43±0.08b	17.01±0.41a
FRAP [μmol Trolox/g d.m.]	3.73±0.15b	9.74±0.78a
PCL ACW [μmol Trolox/g d.m.]	0.50±0.02b	1.95±0.05a

372

373 OF, oat flour; BF, buckwheat flour. Data expressed as mean±standard deviation (n=3).
374 Different letters within the same row indicate statistically significant differences at $p<0.05$ in
375 LSD Fisher test.

376

Table 2 The glycaemic index and antioxidant capacity (before and after *in vitro* digestion) of analysed breads.

		O	OB20%	OB50%	OB80%	B
Glycaemic index [%]		69±0.6c	70±0.4c	72±0.1a	71±0.3b	72±0.4a
TPC [mg GAE/g d.m.]	before digestion	0.14±0.01e	0.38±0.02d	0.82±0.01c	1.15±0.02b	1.27±0.02a
	after digestion (soluble fraction)	2.8±0.12e	3.48±0.12d	4.17±0.06c	4.57±0.07b	5.6±0.01a
	after digestion (insoluble fraction)	0.73±0.01e	1.12±0.04c	1.21±0.01d	1.39±0.03b	1.62±0.02a
ABTS [μmol Trolox/g d.m.]	before digestion	1.98±0.14e	2.95±0.15d	4.02±0.34c	5.91±0.18b	6.31±0.22a
	after digestion (soluble fraction)	68.14±0.77d	77.01±0.66c	89.32±1.41b	91.40±0.09b	98.04±0.23a
	after digestion (insoluble fraction)	12.50±0.04d	17.10±0.25c	21.82±0.19b	23.93±0.29a	24.78±0.63a
FRAP [μmol Trolox/g d.m.]	before digestion	2.54±0.08d	3.57±0.08c	3.78±0.21c	4.65±0.31b	5.55±0.36a
	after digestion (soluble fraction)	4.54±0.02e	7.23±0.12d	9.44±0.04c	11.36±0.08b	12.88±0.02a
PCL ACW [μmol Trolox/g d.m.]	before digestion	0.49±0.02d	0.88±0.09c	1.33±0.16b	2.22±0.13a	2.38±0.26a
	after digestion (soluble fraction)	1.36±0.10d	2.67±0.03c	4.46±0.19b	4.57±0.09b	5.86±0.04a

O: oat bread (100% of oat flour); B: buckwheat bread (100% of buckwheat flour); OB20%: oat-buckwheat bread (80% of oat flour and 20% of buckwheat flour); OB50%: oat-buckwheat bread (50% of oat flour and 50% of buckwheat flour); OB80%: buckwheat-oat bread (20% of oat flour and 80% of buckwheat flour). Data expressed as mean±standard deviation (n=6). Different letters within the same raw indicate statistically significant differences at $p<0.05$ in LSD-Fisher test.